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Antibacterial Activity of the Ethanolic Bark Extract of *Syzygium cumini* on *Escherichia coli* Recovered from Surface Waters Used in Ishaka Municipality

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ABSTRACT

The aim of this research was to investigate the antibacterial activity of the ethanolic bark extract of *Syzygium cumini* on *Escherichia coli* recovered from surface waters used in Ishaka municipality. The study involved the collection of surface water samples and the isolation of *E. coli* strains from these samples. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on (ciprofloxacin, amoxicillin, ceftriaxone, and gentamicin) while the resistance and intermediate isolate were subjected to the antibacterial effect of ethanolic bark extract of *Syzygium cumini*. The antibacterial activity of the *S. cumini* bark extract was evaluated using the disc diffusion method and the zone of inhibition was measured. The results showed that the surface waters sampled had a high total bacterial count, indicating potential contamination. The *E. coli* isolates were found to have varying levels of sensitivity and resistance to the antibiotics tested, with ciprofloxacin and amoxicillin showing the highest resistance rates. The *S. cumini* bark extract demonstrated good antibacterial activity against some *E. coli* strains, particularly BI1 and RI1, but was less effective against LI1, LI4, and rl1. Overall, the study suggests that the ethanolic *S. cumini* bark extract has potential as an alternative or complementary antibacterial agent against *E. coli* in surface waters, but further investigation is required to fully assess its efficacy and safety.

Keywords: *Syzygium cumini*, Surface waters, Antibacterial agent, Zone of inhibition, Antibacterial resistance, *Escherichia coli.*

INTRODUCTION

Syzygium cumini (L.) Skeels, commonly known as Jamun, is a plant species belonging to the family Myrtaceae. The bark of *S. cumini* has been used in traditional medicine for the treatment of various diseases including diabetes, diarrhea, and dysentery (Chandra et al., 2017). Several studies have reported the antibacterial activity of *S. cumini* extracts against various bacterial strains [2]. However, there is limited research on the antibacterial activity of *S. cumini* bark extract on *Escherichia coli* recovered from surface waters. *Escherichia coli* is a gram-negative bacterium commonly found in the intestines of humans and animals [3-6]. However, some strains of *E. coli* can cause serious illnesses such as urinary tract infections, meningitis, and foodborne illnesses [7-9]. The presence of *E. coli* in surface waters used for drinking, washing, and recreation can pose a serious health risk to humans [10-12]. Therefore, there is a need to develop effective methods to control E. coli contamination in surface waters. Surface water is water that emerges at the surface without a perceptible [13]. Water is an essential component of every life [14]. Among the pathogens disseminated in water sources were enteric pathogens such as enterotoxigenic *E. coli.* Virulence genes of *E. coli* correlate to their pathogenic nature causing severe morbidity and mortality in humans and animals [15]. Current allopathic medications for the management of various diseases pose some adverse effects which sometimes compel patients to quit the use of such medications [16-18]. Antibacterial medications are no exemption to this challenge coupled with resistant pathogens and excessive cost, especially in rural communities of some developing

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countries, thus the need for alternatives has created a significant challenge [19-21]. The study aims at the potential of the ethanolic bark extract of *Syzygium cumini* to inhibit the growth of *E. coli*, which is known to cause diseases in humans and animals when ingested. So the research seeks to explore potential natural sources of antibacterial agents for public health applications, considering the increasing prevalence of antibiotic-resistant bacteria and the need for effective and safe alternatives to the conventional antibiotics.

METHODOLOGY

Study Design

This study employed an experimental study design in which the effect of ethanolic bark extract of *Syzygium cumini* on the selected isolate of *E. coli* was determined, antimicrobial susceptibility profile was determined.

Area of Study

The study was conducted in Ishaka, Bushenyi district where water was collected from the selected areas, and analysis was done in the institutional biomedical research laboratory of Kampala internal university – western campus. Bushenyi is bordered by Rubirizi district to the northwest, buhweju district to the northeast, Sheema district to the east, mitooma district to the south, and Rukungiri district to the west. The largest town in the district, Ishaka, is located 75 kilometers, by road, northwest of Mbarara, the largest city in the Ankole sub-region. The coordinates of the districts are 00 32s, 30 11E.

Plant collection and authentication

The bark of *Syzgium cumini* was collected from the wild at Pandwong division, kitgum municipality, Kitgum district. The plant A herbarium specimen was prepared by collecting the bark which was kept and transported in a cool dry paper bag and taken for collection and identification in the herbarium. Identification was confirmed by Makerere herbarium (Olivia W. Maganyi) and voucher specimen number OC001 was given.

Plant extraction

The extraction method described by Alum *et al.* [22] was adopted. The bark was washed and dried at room temperature on the benches at the IBR Laboratory for 15 days. The bark was then crushed into a fine powder using a mortar and pestle. The extract was then prepared by the addition of 5g of dried powder into 50 ml of 70% ethanol in a conical flask and placed on the shaker for 3 days. It was then filtered by use of filter paper and the filtrate was concentrated on the rotary evaporator. Later, it was put in the hot oven overnight and the dry extract was obtained and kept in the refrigerator for further studies.

Target samples

The target samples were the water from different points in Ishaka (ENVIRONMENTAL SOURCE)

Sampling technique and procedures

The different points where the samples were collected were selected using a simple random sampling technique (SRS). Using this method, every point of water located in Ishaka, Bushenyi district, had an even chance and likelihood of being selected, and its water was used as the sample.

Collection of samples

A sterile dry container was used to collect the water samples. The sterile containers were given a number based on each water point and were only opened during sample collection. The collected water samples were placed in a cooler box from the collection areas to the IBR laboratory for analysis.

Bacterial isolation and identification

Water samples were collected from 20 selected water sources from Ishaka, Bushenyi and labeled as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,20. The water samples were collected between March and April in sterile glass bottles, packed in an ice box, and transported to the IBR laboratory, KIU-western campus, for culture within 3 hours of collection. The water samples were homogenized and pre-enriched in 5ml of peptone water for 2 hours, subcultured onto prepared hicrome agar media were incubated at 37℃ for 24hrs for *E. coli*. Discrete colonies from the enumeration with a blue coloration on Hicrome media were considered presumptive E. coli. These isolates were then stored in 25% glycerol stock and kept in the refrigerator.

Enumeration of total bacterial count

The total bacterial counts of the spring water collected were determined by the pour plate technique using nutrient agar media. Serial dilution (10-1 to 10-5) of the water samples was made in distilled water. And aliquots of 0.5mls were added into each pre-labeled petri dish with a cooled number according to the type of juice. A sterilized nutrient agar cooled to 45℃ was added to each of the peri-dishes containing the sample and left on the bench to solidify at room temperature. The agar plates were then incubated at 37℃ for 18-24 hours. For 18-24hours. After incubation, all plate colonies were counted for all different dilutions made, and the colony forming units per ml were calculated using a standard formula as shown below to obtain the total viable count.

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$$\text{Cfu/ml} = \text{colony counted} \times \frac{1}{dl} \times \frac{1}{vol\ plated\ (ml)}$$

Antimicrobial susceptibility studies

The antimicrobial susceptibility tests were performed using the Kirby Bauer disk diffusion technique as described by Hudzicki [23] using commercially available disks on Mueller Hinton agar plates. Antibiotic disk viability was controlled using *E. coli* ATCC25922. The agar was poured to a uniform depth of 4mm and allowed to cool and solidify according to clinical and laboratory standards institute and international guidelines 2021. A 0.5 McFarland turbidity standard was prepared according to the method described by Kirk. A solution with 9.95ml of 1% chemically pure sulphuric acid was mixed with 0.05ml of 1.175% barium chloride to form a barium sulfate precipitate which cause turbidity. This standard was used to adjust the turbidity of the bacteria inoculums for the antimicrobial susceptibility test. Well-isolated single colonies were transferred to a clean dried tube containing sterile saline, mixed together and the suspensions were compared to 0.5McFarland standard. After the turbidity of the inoculum is adjusted, a sterile cotton swab was dipped into the suspension, and pressed firmly against the inside wall of the tube to avoid excess inoculum; the swab was then streaked over the surface of the medium 3 times rotating the plate after each application to ensure even distribution and was allowed to stand at room temperature for 10minutes. Antimicrobial disks (amoxicillin, ciprofloxacin, ceftriaxone, gentamycin) commonly used and containing specified concentrations in micrograms were placed on the agar plates after 10 minutes using a pair of sterile forceps and then gently pressed down on the agar to ensure contact. The plates were inverted and incubated at a temperature of 37°C for 24 hours. After incubation, the diameter zone with complete inhibition was measured using a ruler in millimeters and interpreted as sensitive, resistant, and intermediate according to clinical laboratory standards institute criteria 2021.

Determination of zone of inhibition

A fresh medium of Muller Hinton agar was prepared, sterilized, and validated for sterility overnight by incubating at 37°C. Using a sterile swab, a small amount of the bacterial culture on the hi-chrome medium was transferred to the prepared Muller Hinton agar, it was spread uniformly across the whole agar. Wells were punched onto the agar plates, then different concentration values of the extract were labeled at the back side of the plates and the corresponding concentrations of the extract shall be added, about 50µl in each well [24]. Likewise, different concentrations of a standard drug in the form of disks were prepared through dilutions in normal saline. In the same manner, the drug concentrations are also labeled on the plates and an equal volume as that of the extracts will also be added to the designated wells for the drug. The negative control was normal saline and the positive control was the standard drug. This setup was transferred into the incubator set at 37°C and left to stand overnight for up to about 24 hours. The plates were then removed, and using a screw gauge, the respective zones of inhibition of the standard drug and the extracts were measured and recorded in millimeters.

Measurement of mic

The same concentrations of the extracts as those prepared above shall be used here. A nutrient broth was prepared by boiling followed by autoclaving. A suspension of the *E. coli* bacterial strains was prepared in different test tubes to act as an equivalent of a Mc Farand solution. Bacteria were suspended in normal saline and their optical density was read using a spectrophotometer, bacteria were added into the test tube until the optical density is between 0.3-0.4. 100µl of the nutrient broth was added to each well of the microtiter plate, up to about 10 wells in total. Then, 100µl of a freshly prepared definite concentration of the drug and the extract was prepared. 100µl of each was then added to the first well respectively following the labels. It was properly mixed and transferred to the next well, 100µl as well. This serial dilution shall go on until the last well where 100µl of the drug and the extract was pipetted and discarded [25]. Positive control was the broth and bacteria alone, without the extract or drug. The negative control was the broth alone. Then, 100µl of the bacterial suspension was added in each of the wells, and incubated for 24 hours. Resazurin reagent, about 100µl was then added to all the wells and incubated for about one hour. A color change to pink signifies bacterial growth, whereas no color change signifies no growth. The Concentration of the extract/ drug just before which growth appeared was identified and recorded as the MIC.

Measurement of MBC

This was performed after the MIC. Using a sterile wire loop, the solutions in the wells with no visible growth of bacteria after 1 to 2 days of incubation were introduced on freshly prepared Muller-Hinton agar by the streak plate method and were incubated at 37°C for 1 to 2 days. The plates were checked for any colony growth. The least concentration of the extract which had no visible colony growth was considered as the minimum bactericidal concentration [25].

Statistical analysis

Data obtained from this study were captured into Microsoft Excel (version 2016), GraphPad Prism, and SPSS and results were presented in the form of tables and graphs.

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RESULTS

Total bacterial count Enumeration of total bacterial load

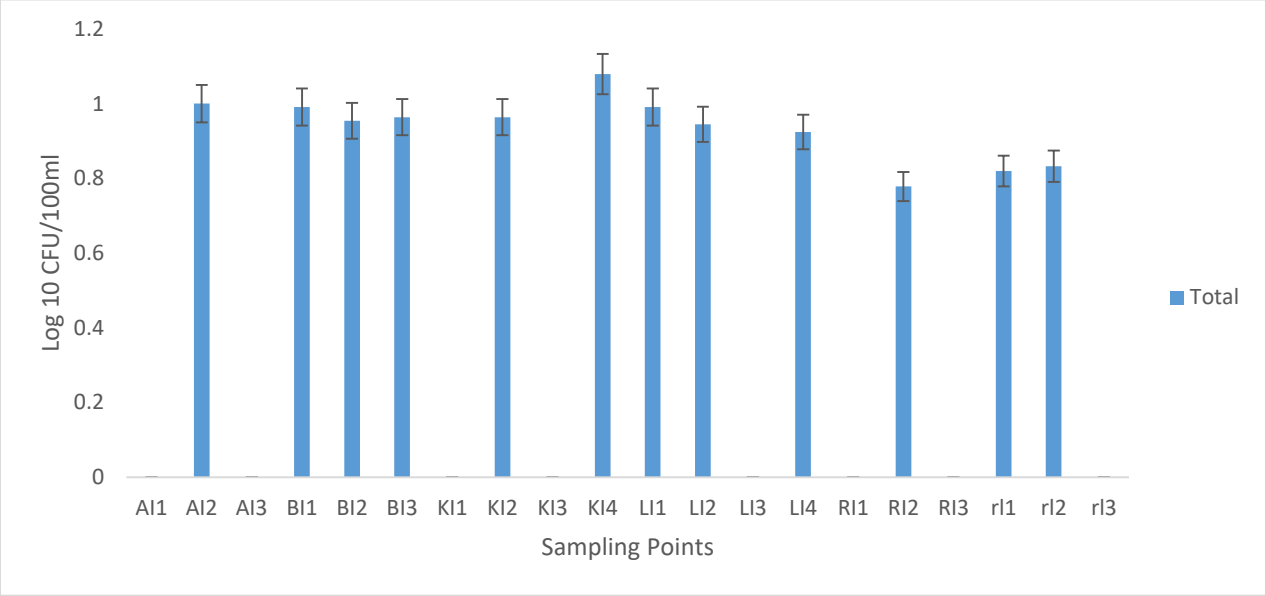

Figure 1: showing the total bacterial count of log10 CFU of surface water from the location in Ishaka municipality.

The *E. coli* count was significantly higher in the bar graph above indicating the following surface water coded as A12, B11, B12, B13, K12, K14, L11, L12, L14, r11, r12. K12 has the highest bacterial load of 7.96 Log 10 CFU/ml to be having a higher total bacterial count.

Antimicrobial susceptibility pattern

Table 1: Antimicrobial susceptibility pattern of presumptive isolates from the surface water from the selected areas in Ishaka municipality

TABLE 1 (SUSCEPTIBILITY PATTERN)				
presumptive isolates	Ciprofloxacin 5µg	Amoxicillin 10µg	ceftriaxone 30µg	Gentamycin 10µg
LI1	R	R	I	R
Ll2	S	S	S	S
LI4	R	R	R	R
AI2	S	S	R	R
KI2	S	S	S	S
KI4	S	S	S	S
BI1	R	R	R	R
BI2	S	S	S	S
BI3	S	S	S	S
RI1	R	R	R	R
rl1	R	R	R	R
rl2	S	S	S	S
	S = 58.3%, I = 0%, R = 41.7%	S = 58.3%, I = 0%, R = 41.7%	S = 50%, I = 8%, R = 42%	S.50.0%, I. 0%, R.50.0%

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Analysis based on (CLSI, 2021)
Zone of inhibition. S = Susceptibility, l = intermediate, R= resistance.
The table above indicates the sensitivity of antibiotics to the presumptive isolate of E coli and it shows that 58. 3% sensitive to amoxicillin, 50% sensitive to ceftriaxone, and 50% sensitive to gentamycin. On resistance, 41.7% was resistant to ciprofloxacin and amoxicillin, 42% was resistance to ceftriaxone, 50% was resistance to gentamycin, and 8% was intermediate to ceftriaxone. The presumptive isolates which show resistance to ciprofloxacin were 5 (41.67). amoxicillin was 5(41.67), resistance to ceftriaxone was 5(41.67). while those resistances to gentamycin were 6 (50) of the class of aminoglycoside.

Antibacterial activity of S. *cumini* on resistant and intermediate isolates of E *coli*.

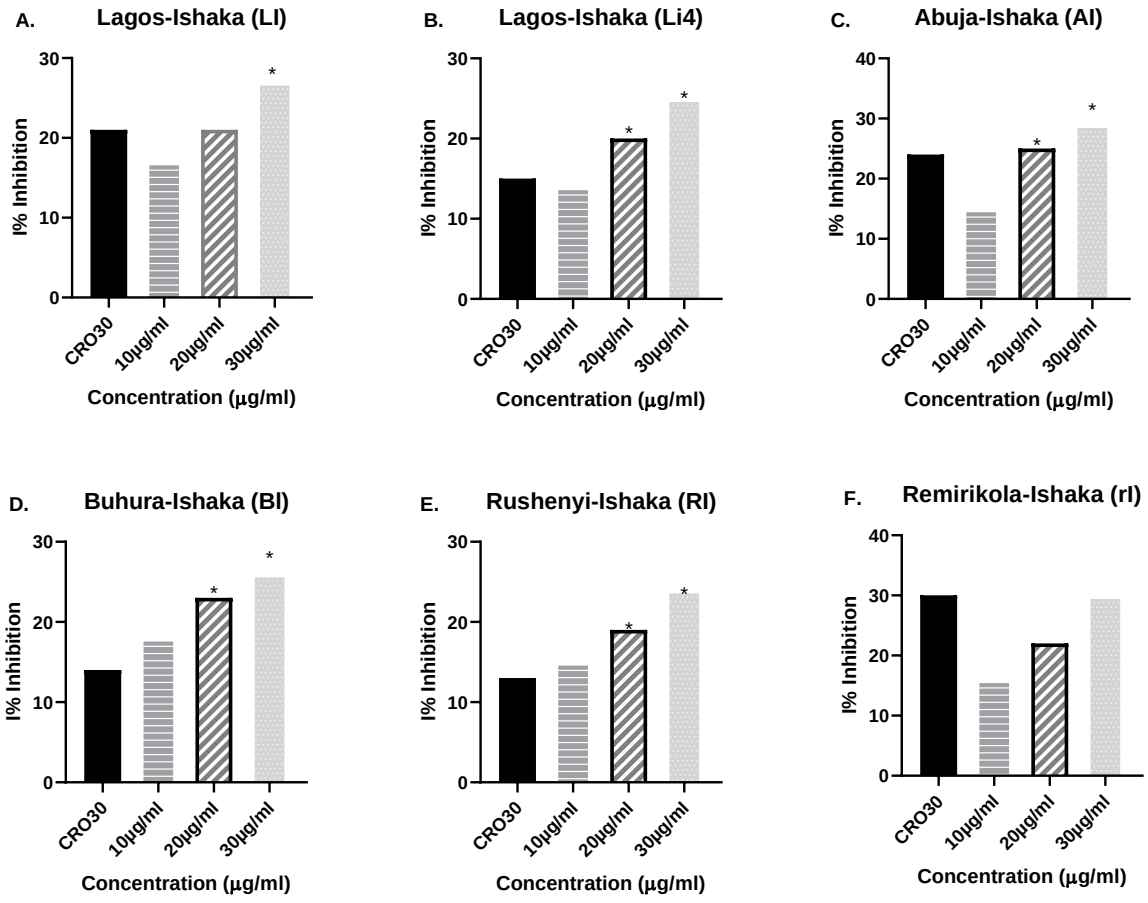

Figure 2 A-F: Antibacterial activities of *Syzygium cumini* ethanolic extract on a resistant and intermediate isolate of *E. coli* compared to ceftriaxone.
X P < 0.05 CRO30 versus *Syzygium cumini* ethanolic extract of different doses.
A: 30ug/ml significantly inhibits the growth of *Escherichia coli*; B: 30 ug/ml significantly inhibits the growth of *Escherichia coli*; C: 30ug/ml significantly inhibits the growth of *Escherichia coli*; D: 30ug/ml significantly inhibits the growth of *Escherichia coli;* E: 30ug/ml significantly inhibit the growth of *Escherichia coli;* F: 30 ug/ml significantly inhibit the growth of *Escherichia coli*, The result presented in Figure 3 shows the antibacterial activity of the ethanolic extract of *Syzygium cumini* and ceftriaxone against various isolates.

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DISCUSSION

This study examines the antibacterial activity of the ethanolic bark extract of *Syzygium cumini* on *E. coli* recovered from surface waters used in Ishaka Municipality. The findings on *E. coli* recovered from surface waters suggest that 55% of the surface water samples coded as A12, B11, B12, B13, K12, K14, L11, L12, L14, r11, and r12 have higher total bacterial counts. The study also identified several surface water sources with high *E. coli* counts, including A12, B11, B13, K12, K14, L11, L12, L14, r11, and r12. *E. coli* is an indicator organism for fecal contamination of water sources and its presence indicates a potential risk of waterborne infections [7-9]. The high *E. coli* counts in these water sources suggest that they may be contaminated with fecal matter, possibly from human or animal waste. The water source K12 had the highest bacterial load of 7.96 Log 10 CF/ml, indicating a very high bacterial abundance. This finding suggests that the K12 water source is highly contaminated, and poses a significant public health risk. The high bacterial load could be due to several factors, such as poor sanitation, inadequate infrastructure, and human activities in the surrounding area. Total bacterial count is a measure of the number of bacterial cells present in a given sample [26, 27]. High bacterial counts in surface water can indicate poor water quality and potential health hazards [11]. Major enterocyte-infecting bacteria are currently thriving beyond the expectation even as various control measures are being put in place to combat enteric infections [28]. The CLSI guidelines were used to interpret the results obtained from the antimicrobial susceptibility testing. The results were obtained from the antimicrobial susceptibility testing. The results showed that the isolates were resistant to commonly prescribed antibiotics such as ciprofloxacin, amoxicillin, and ceftriaxone, while half of the isolates were resistant to gentamycin. Similar findings have been reported in previous studies. A study conducted by Owoseni and Okoh [29] found that *E. coli* isolates from surface water were resistant to ciprofloxacin, amoxicillin, and ceftriaxone, while half of the isolates were resistant to gentamicin. Similar findings have been reported in previous studies. The emergence of antibiotic-resistant bacteria was a consequence of the inappropriate and excessive use of antibiotics in human and animal health, agriculture, and aquaculture [30]. It is, therefore, essential to control the use of antibiotics and ensure that they were only prescribed when necessary. It is worth noting that the isolate shows intermediate resistance to ceftriaxone, which means that while it is not fully susceptible to the antibiotic, it is also not fully resistant. This result may indicate that the isolate is developing resistance to this antibiotic and could potentially become fully resistant in the future. The occurrence of antimicrobial-resistant *E. coli* in the environment has been proposed to indicate the presence of other public health and environmentally significant antimicrobial-resistant bacteria [12]. The high resistance may be due to overuse of antibiotics, over the use of antibiotics in veterinary. The sensitivity test to commonly used antibiotics against the presumptive isolates of *E. coli* shows high resistance to ceftriaxone and gentamicin compared to ciprofloxacin and amoxicillin. The results show that at a dose of 30ug/ml, the ethanolic extract of *S. cumini* significantly inhibited the growth of *E. coli* isolates A, B, C, D, and E, which were either resistant or intermediate to ceftriaxone. However, the extract did not significantly inhibit the growth of *E. coli* isolates F. These findings suggest that *Syzygium cumini* ethanolic extract has antibacterial activity against some *E. coli* isolates that are resistant or intermediate to ceftriaxone, which is a commonly used antibiotic for the treatment of bacterial infections. The results also suggest that the antibacterial activity of *Syzygium cumini* ethanolic extract may vary depending on the bacterial strain. The results indicate that the plant extract had good activity against B11 isolates, with longer zones of inhibition compared to ceftriaxone. This suggested that *S. cumini* has potential as an antibacterial agent against these isolates. However, for LI1, LI4, and rl1 isolates, ceftriaxone had better activity than *S. cumini*. This may be due to the fact that ceftriaxone is a broad-spectrum antibiotic that is effective against a wide range of bacteria, while *S. cumini* may have a more limited spectrum of activity. It is also possible that the isolates that were more susceptible to ceftriaxone may have mechanisms of resistance that are not affected by the plant extract.

CONCLUSION

This investigation indicates that there is a high level of bacterial count in the surface water used in Ishaka municipality. So those water sources are not safe for human consumption. And will also increase the risk of water-related disease in Ishaka. The results of this study emphasize the importance of antibiotic stewardship and responsible use of antibiotics to prevent the emergence of antibiotic-resistant bacteria. It is also important to continue monitoring antibiotic resistance patterns in bacterial isolates to inform appropriate treatment strategies and prevent the spread of resistant strains. These results suggest that the ethanolic bark extract of *Syzgium cumini* may be a potential alternative to conventional antibiotics for the treatment of certain bacterial infections.

RECOMMENDATION

The study has created the baseline for water surveillance. This study suggests and recommends that all surface water should be regularly treated, and disinfected to ensure safe water for the Ishaka community. Health education

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on proper hygiene and sanitation among the Ishaka community. Additionally, it is important to investigate the potential sources of contamination and take appropriate actions to address any issues identified.

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